DEEP-FREEZING FLOWERS FOR LABORATORY INSTRUCTION IN SYSTEMATIC BOTANY

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Electrical deep-freezing machines are widely used in American homes for the preservation of frozen foods. Their general availability and economical operation suggested the possibility of their use to preserve fresh botanical material for use in laboratory instruction. Apparently a number of persons have been similarly impressed by such possibilities and at least two short notes, Baker (1949) and Harrington (1950), have appeared indicating success in this direction. However, in 1948, when I began preparations for a test run on a variety of different flowers that might appropriately be used in teaching taxonomic botany, no guiding information was available. Now, a fairly wide selection of angiosperm flowers has been used in laboratory instruction after having been preserved in a deep-freezer for periods of eight to fourteen months. For teaching purposes, the superiority of these materials over pickled or dried specimens is very striking. Not only are the shapes and color of most flowers kept intact, but even the characteristic odors are preserved in many instances. The results obtained, I believe, justify the prediction that a deep-freezer will become an indispensable adjunct to most taxonomic laboratories and may be found very useful for instruction in other branches of botany. It is particularly needed in areas where the seasons preclude easy access throughout the year to flowering plants growing in the wild. But even in the warmer climates, the deep-freezer may be used to keep material for introduction to the students in a more logical sequence than is often possible from naturally growing plants.

The advantages of frozen flowers are similar to those of fresh material over dried or pickled specimens. The student is permitted to formulate a three-dimensional concept of the flowers studied and may easily obtain an accurate picture of the positional relationships of the flower parts. When first removed from the freezer, the flowers are frozen stiff. As thawing takes place, they become less turgid and ultimately wilt. It has been found effective to pass the specimens to the students in the frozen

condition so that preliminary observations may be made immediately. Dissections follow as the material becomes sufficiently pliable to allow manipulation.

Containers.—Some attention has been given to methods of packaging, in an attempt to find the most effective procedures and the most satisfactory containers for the purpose. Two types of stiff wax-impregnated paper containers were used. One type was cylindrical in shape, with a tight-fitting lid. This type was of pint ($3\frac{3}{8}''$ in diam.) and quart ($4\frac{1}{4}''$ in diam.) size. These are designated "round unsealed" in Table I. The material was placed directly into these containers, with no additional sealing except for the tight-fitting lid. The other type of container was a rectangular waxed paper carton of pint or quart size, together with an individual removable inner pliofilm bag. These are designated "rectangular sealed" in Table I. In using the latter, specimens were sealed inside the pliofilm bag by using heat to meld the edges together. The sealed bag was then placed inside the paper carton for storage.

Preparation of Material.—In preliminary trials, it was found that wilted specimens did not become turgid when placed in the deep-freezer. They froze in the wilted condition and were unusable. To prevent wilting, specimens were collected in a vasculum. If any wilting occurred, the cut ends of the stems were put in water until turgidity was restored. Flowers, or flower-clusters snipped from larger specimens, were put loosely into containers that in turn were placed immediately into the deep-freezer machine. The material was not pre-frozen in a special low-temperature compartment.

Flowers collected when rain was falling, or otherwise wetted before freezing, were in general unsatisfactory. Ice crystals commonly formed over the tissue-surfaces, or the parts became embedded in large or small pieces of ice. This made it difficult to handle the specimens for class distribution, although in most instances they were not completely ruined.

Sealing containers.—The commonest failure was caused by the drying-out of specimens. The sealed pliofilm bags were slightly more effective in preventing excessive drying than were the cylindrical containers, but more work was involved in putting up the material. Manufacturers recommend the use of an ordinary

clothes iron for sealing the pliofilm bags, but I found a hot incandescent electric light bulb to be equally effective and much less trouble. A half-inch margin at the open end of the bag was pressed momentarily against a lighted, firmly anchored light bulb. Sealing was immediate. The rectangular cartons, each holding a pliofilm bag, are easy to stack efficiently in the freezer and require much less room than comparable storage space provided by the cylindrical containers. The principal reason for using small individual cartons was to prevent crushing and facilitate finding the particular species when ready for use. However, if space is at a premium, I see no reason why a number of sealed pliofilm bags should not be put together in a larger carton than was used in these trials. In favor of the cylindrical cartons, it should be stated that they are easier to fill and material may be removed at intervals without breaking a permanent seal. These are items of convenience not to be too heavily discounted. An important consideration is the amount of unfilled space in a bag or carton. In those instances where the amount of material left the available space less than half-filled, there was more frequent drying-out than when the container was completely filled. This varied somewhat with the nature and succulence of the specimens but was sufficiently consistent to form the basis for a definite recommendation that the containers be full or nearly full for best results. On the other hand, pressing or packing the specimens too closely is not a good practice, for they freeze together and become distorted in shape.

Temperature.—No special mechanism for temperature control, other than that already on the General Electric deep-freezer, was used. The regulator was set to keep the temperature below − 10° C. Actually, as set, the regulator permitted a range from − 10° to − 20° C. (+ 18° to − 4° F.). This temperature range seemed satisfactory for the purpose and was not altered during the test.

Results.—The results of deep-freezer trials on a variety of plants are presented in tabular form below. We have attempted to give an overall estimate of the usefulness of the method for each species, in the column "satisfactory vs. unsatisfactory for laboratory use." There was no attempt to try material from every family of the Angiosperms, those species most easily

available being used. However, the range of material is sufficiently wide to demonstrate the effectiveness of the method. The results are really self-evident, but an examination of the table points up the fact that either type of container is satisfactory for the purpose. By far the largest majority of flowers showed good color and texture. The most common wilting time, after the material was taken from the freezer and exposed to the open air of the laboratory, was between 10 and 60 minutes. In general, there was ample time for the student to make proper preliminary observations before wilting set in, and in only a few cases was wilting so rapid that the material was unsatisfactory for laboratory use.

To give the interested person the benefit of my experience, the following items in connection with procedure are noted:

(1) Be sure the specimens are neither wilted nor wet when placed in the deep-freezer.

(2) Prevent drying of the specimens by placing them in sealed or semi-sealed containers.

(3) The bulk of the specimens should exceed half of the total space inside the container for best results.

(4) In order to permit easy removal and separation of the material, do not pack it too tightly.

(5) In general, relatively small containers (i. e. half-pint, pint or quart size) are preferable to larger ones because they eliminate crushing due to the weight of a large mass of material. However, this depends in part upon the size and type of material being frozen, as well as the nature of the prepared specimen. I have usually frozen only the flowers and fruits, and these have been supplemented with dried specimens for teaching purposes.

(7) The temperature of the deep-freezer should be below the freezing point of water at all times. Fluctuations from $-10°$ to $-20°$ C. ($+18°$ to $-4°$ F.) were not harmful to the material and allowed for a considerable margin of safety in connection with the operation of the machine.

LITERATURE CITED

BAKER, GLADYS E. 1949. Freezing Laboratory Materials for Plant Science. Science **109:** 525.

HARRINGTON, H. D. 1950. Preserving Flowers by Freezing. Turtox News **28:** 51.

TABLE I

Name	Container		Natural Flower Color		Texture of Flower Parts		Wilting time in open air			For lab. use		Remarks
	Rect. Sealed	Round Unsealed	Good	Poor	Good	Poor	<10 min.	10-60 min.	1-3 hrs.	Satis-factory	Unsatis-factory	
Gramineae												
Agropyron repens (L.) Beauv.		×	×		×				×	×		
Anthoxanthum odoratum L.	×		×		×				×	×		
Danthonia spicata (L.) Beauv.		×	×		×				×	×		
Festuca rubra L.		×	×		×				×	×		
Cyperaceae												
Carex stricta Lam.	×		×		×			×		×		
Araceae												
Arisaema Stewardsonii Britt.	×		×		×			×		×		
Calla palustris L.		×	×	×	×	×					×	Thawed and refrozen.
Symplocarpus foetidus (L.) Nutt.	×		×		×			×		×		
Commelinaceae												
Tradescantia virginiana L.		×	×		×		×			×		Wilts rapidly; dissected under water.
Juncaceae												
Juncus effusus L.		×	×		×				×	×		
Luzula campestris (L.) DC.	×		×		×				×	×		
Liliaceae												
Maianthemum canadense Desf.	×		×		×			×		×		Container open; material somewhat dry but good.
Polygonatum pubescens (Willd.) Pursh.	×		×		×			×		×		Bag broken; material still good.
Uvularia perfoliata L.	×		×		×			×		×		
Orchidaceae												
Cypripedium acaule Ait.	×		×		×			×		×		
Salicaceae												
Salix humilis Marsh, var. microphylla (Anderss.) Fern.	×		×		×				×	×		
Myricaceae												
Comptonia peregrina (L.) Coult.		×	×		×			×		×		
Corylaceae												
Alnus rugosa (Du Roi) Spreng.	×		×		×			×		×		
Betula populifolia Marsh	×		×		×			×		×		
Ostrya virginiana (Mill.) K. Koch	×		×		×			×		×		
Fagaceae												
Fagus grandifolia Ehrh.	×		×		×			×		×		
Quercus velutina Lam.	×		×		×				×	×		
Santalaceae												
Comandra umbellata (L.) Nutt.		×	×		×			×		×		

TABLE I—Continued

Name	Container		Natural Flower Color		Texture of Flower Parts		Wilting time in open air			For lab. use		Remarks
	Rect. Sealed	Round Unsealed	Good	Poor	Good	Poor	< 10 min.	10-60 min.	1-3 hrs.	Satis-factory	Unsatis-factory	
Aristolochiaceae												
Asarum canadense L.	×		×		×				×	×		Thawed and refrozen but still very good.
Polygonaceae												
Polygonum persicaria L.		×	×		×			×		×		
Rumex acetosella L.		×	×		×			×		×		
Phytolaccaceae												
Phytolacca americana L.		×	×		×			×		×		
Caryophyllaceae												
Lychnis alba Mill.	×		×		×		×			?		Wilts rapidly; difficult to dissect.
Saponaria officinalis L.		×	×		×			×		?		Difficult to dissect.
Silene cucubalus Wibel		×	×		×					×		Wilted on removal.
Ranunculaceae												
Anemone quinquefolia L.	×		×		×			×		×		Flowers rapidly brown on thawing.
Aquilegia canadensis L.	×		×		×		×			×		Flowers retain odor.
Caltha palustris L.	×		×		×		×			×		Quite soft when thawed.
Ranunculus acris L.		×	×			×					×	Wilted at time of removal.
Ranunculus bulbosus L.		×	×		×			×		×		
Berberidaceae												
Berberis vulgaris L.	×		×		×				×	×		
Podophyllum peltatum L.		×	×		×				×	×		
Magnoliaceae												
Liriodendron tulipifera L.		×	×		×			×		×		Retain shape well.
Magnolia denudata Desrouss. (fls.)	×		×		×			×		×		Characteristic odor present.
Magnolia denudata Desrouss. (frt.)		×			×				×	×		
Lauraceae												
Sassafras albidum (Nutt.) Nees var. molle (Raf.) Fern.	×		×		×			×		×		
Papaveraceae												
Chelidonium majus L.	×		×		×		×			?		Difficult to dissect when wilted.
Dicentra canadensis (Goldie) Walp.		×	×		×			×		×		
Cruciferae												
Barbarea vulgaris R. Br. (fls.)	×		×		×			×		×		Wilts only partially when thawed.
Barbarea vulgaris R. Br. (frt.)		×	×		×			×		×		Wilts only partially when thawed.
Brassica campestris L.		×	×		×			×		×		Wilts only partially when thawed.
Capsella bursa-pastoris (L.) Medic.	×		×		×			×		×		Wilts only partially when thawed.
Dithyrea Wislizenii Engelm.		×	×		×			×		×		Wilts only partially when thawed.

TABLE I—Continued

Name	Container		Natural Flower Color		Texture of Flower Parts		Wilting time in open air			For lab. use		Remarks
	Rect. Sealed	Round Unsealed	Good	Poor	Good	Poor	<10 min.	10-60 min.	1-3 hrs.	Satis-factory	Unsatis-factory	
Saxifragaceae												
Philadelphus inodorus L.		×		×		×		×		×		Dried in container.
Saxifraga virginiensis Michx.		×	×		×			×		×		
Hamamelidaceae												
Fothergilla major (Sims) Lodd.	×		×		×				×	×		
Rosaceae												
Crataegus (hybrid)	×		×		×			×		×		Browns rapidly.
Potentilla simplex Michx.	×		×		×			×		×		Wilts only partially.
Prunus pensylvanica L.	×		×		×			×		×		
Pyrus melanocarpa (Michx.) Willd.	×			×	×			×		×		Thawed and refrozen before use.
Rhodotypos scandens (Thunb.) Mak.		×	×		×			×		×		Wilts only partially.
Rubus sp.		×	×		×			×		×		Wilts only partially.
Leguminosae												
Cercis canadensis L.	×		×		×			×		×		
Cladrastis lutea (Mich. f.) K. Koch.	×		×		×				×	×		
Robinia pseudoacacia L.		×	×		×				×	×		
Trifolium pratense L.		×	×			×				×		Partially dried in container.
Wisteria sinensis Sweet	×		×		×			×		×		
Geraniaceae												
Geranium maculatum L.	×		×			×		×		×		Slightly wilted on opening; petals fall easily.
Oxalidaceae												
Oxalis stricta L.	×		×			×	×				×	Very soft when thawed; difficult to dissect.
Polygalaceae												
Polygala paucifolia Willd.	×		×		×			×		×		
Euphorbiaceae												
Euphorbia cyparissias L.	×		×		×			×		×		Soft but easily dissected.
Celastraceae												
Celastrus scandens L.		×	×		×			×		×		
Aceraceae												
Acer negundo L.	×		×		×			×		×		
Acer platanoides L.	×		×		×			×		×		
Hippocastanaceae												
Aesculus glabra Willd.	×		×		×			×		×		
Vitaceae												
Vitis Labrusca L.		×	×		×				×	×		

TABLE I—Continued

Name	Container		Natural Flower Color		Texture of Flower Parts		Wilting time in open air			For lab. use		Remarks
	Rect. Sealed	Round Unsealed	Good	Poor	Good	Poor	<10 min.	10-60 min.	1-3 hrs.	Satis-factory	Unsatis-factory	
Tiliaceae												
Tilia americana L.		×	×		×				×	×		
Malvaceae												
Hibiscus syriacus L.	×		×		×				×	×		Mucilaginous quality preserved.
Guttiferae												
Hypericum perforatum L.		×	×			×			×	×		Partly wilted on opening.
Violaceae												
Viola fimbriatula Sm.	×		×		×			×		×		
Onagraceae												
Oenothera biennis L.		×	×		×			×		×		Top flowers partially dried.
Araliaceae												
Aralia nudicaulis L.	×		×		×			×		×		
Cornaceae												
Cornus alternifolia L. f.		×	×		×			×		×		Stand up well on thawing.
Cornus amomum Mill.		×	×		×			×		×		Stand up well on thawing.
Cornus stolonifera Michx.	×		×		×				×	×		Stand up well on thawing.
Ericaceae												
Pieris floribunda (Pursh) B. & H.	×		×		×				×	×		
Rhododendron canadense (L.) Torr.	×		×		×			×		×		Wilts only partially; pores on anthers good.
Vaccinium angustifolium Ait.	×		×		×			×		×		Flowers change from white to dirty brown.
Vaccinium corymbosum L.	×		×		×			×		×		Flowers brown in ½ hr.
Primulaceae												
Trientalis borealis Raf.	×		×		×		×			?	?	Wilts on exposure.
Styracaceae												
Halesia carolina L.		×	×		×			×		×		Gynoecium difficult to dissect.
Symplocaceae												
Symplocos tinctoria (L.) L'Her.	×		×		×			×		×		
Oleaceae												
Forsythia (hybrid)	×			×		×				×		Wilted on removal; softens excessively.
Syringa vulgaris L.	×		×		×			×		×		
Apocynaceae												
Vinca minor L.	×		×		×		×			×		
Asclepiadaceae												
Asclepias syriaca L.		×	×		×			×		×		

TABLE I—Continued

Name	Container		Natural Flower Color		Texture of Flower Parts		Wilting time in open air			For lab. use		Remarks
	Rect. Sealed	Round Unsealed	Good	Poor	Good	Poor	< 10 min.	10-60 min.	1-3 hrs.	Satis-factory	Unsatis-factory	
Labiatae												
Trichostema dichotomum L.	×		×		×			×		×		
Solanaceae												
Solanum triflorum L.		×	×		×			×		×		
Scrophulariaceae												
Linaria canadensis (L.) Dumont	×		×			×		×		×		Somewhat wilted in container.
Linaria vulgaris Hill	×		×		×			×		×		
Pedicularis canadensis L.	×			×		×		×		×		
Veronica serpyllifolia L.	×		×		×			×		×		
Bignoniaceae												
Catalpa bignonioides Walt.		×	×		×			×		×		
Plantaginaceae												
Plantago lanceolata L.	×		×		×			×		?	?	Difficult to dissect.
Rubiaceae												
Houstonia caerulea L.	×		×		×			×		×		
Caprifoliaceae												
Lonicera Morrowi Gray	×		×		×		×				×	Corolla wilts rapidly to pulpy mass.
Sambucus canadensis L.		×	×		×			×		×		Browns rapidly on thawing.
Viburnum bitchiuense Mak.	×		×		×			×		×		Browns after thawing.
Campanulaceae												
Campanula rapunculoides L.	×		×			×		×			?	Dry in container; too little material.
Lobelia cardinalis L.		×	×		×			×		×		
Compositae												
Antennaria plantaginifolia (L.) Hook.	×		×		×			×		×		
Agoseris cuspidata (Pursh) Raf.		×	×		×			×		×		
Aster ericoides L.		×	×		×			×		×		
Cichorium intybus L.		×	×		×			×		×		
Chrysanthemum leucanthemum L.		×	×		×			×		×		Rays wilt rapidly.
Helianthus annuus L.		×	×		×				×	×		
Senecio aureus L.		×	×			×		×		×		
Silphium perfoliatum L.		×	×		×			×		×		
Solidago sempervirens L.	×		×		×			×		×		
Vernonia noveboracensis (L.) Michx.		×	×		×				×	×		